University of California at Berkeley Department of Nutritional Sciences

1/64

Semi-Annual Progress Report

July 1, 1967 - December 31, 1967

N68-17148

14 pages 1 Code

CR-92980

cat 04

NASA Grant NGR-05-003-118

Title: Nutritional Requirements and Breeding Behavior of Perognathus

Nutritional Requirements:

Following the development of a semipuridied diet (semi-annual report Jan.-June 1967 and manuscript "Development of a Semipurified Diet for the Adult Pocket Mouse (Perognathus)" (in press Journal of Nutrition, March 1968)) we have performed a number of experiements designed to test its adequacy for growth and for maintenance of adult mice and have used the diet for the study of their requirements. Two minor modifications of the diet as listed in the previous report have been made. One was the increase of Gum Arabic USP to 5% of the diet. The lower level of gum made rather soft pellets which the mice ground up during the night and required almost daily feeding. The second mofification concerns the mineral mix. Table 1 gives the composition of the mineral mix. We replaced NaCl by Na-glycerophophate to cut down on the Cl content and replaced K-citrate by KCl because there was no longer any purpose to use citrate. Animals have lived on this modified diet (Gly-7) since June 1967 and are doing well. This includes Pe. longimembris (30-40), Pe. penicillatus (50-70) and Pe. Bailey (4).

I. The following parameters were studied in groups of mice fed different diets in order to assess their adequacy for maintenance of adult mice: Organ weights, carcass composition, serum electrolytes, hematology and histopathology of certain organs. The diets were, I: Mixed seeds; II: semi-purified diet; III: Bell's mouse diet; IV: Bell's mouse diet supplemented with carrots. Diet I is the diet on which the mice seem to thrive indefinitely; diet II is the diet developed by us, diet III is a diet for laboratory mice which has proven to be lethal for Pe. within a short period of time, while diet IV has been shown to maintain weight and general health for periods of 4 months. (For details of diet compositions see previous semi-annual reports.)

Organ weights and carcass composition.

a) The results (Table 2) show that the relative organ weights were the same in all groups. Animals on the semi-purified diet (II) did not differ in body weight and carcass composition from those fed the seeds (I). Animals fed the Bell's diet with or without supplementation with carrots (III and IV) had lower body weights, higher levels of carcass protein and lower levels of carcass fat than did those in groups I and II. The animals on the lethal Bell's diet (III) had less carcass moisture and protein and more fat than those who did relatively well because of the supplementation with carrots(IV). None of these differences were statistically significant. The large standard errors were due to major sex differences in carcass composition. On all diets the females had more fat and less water and protein than did males (Table 3). The lack of a change in body composition when fed the lethal Bell's diet may be explained by a very acute interference with metabolism so that the animals die before major changes can take place. We are now planning to study the effects of the supplementation with carrots on body composition in animals which have been fed this diet for a longer period of time.[1]

b) Serum electrolytes. Because of the exotic mineral requirements of these animals we have measured the concentration of some electrolytes in the circulation. Table 4 shows that there were no differences in the levels of Na and K between animals on adequate (I, II, and IV) or inadequate (III) diets. The levels of Ca and Mg were the same in animals fed the semi-purified diet and seeds. (They have not yet been measureed in animals of groups III and IV.) It is interesting to note that the Mg level in these animals is rather high when compared to those of rats and mice.

c) Histopathology of organs. Gross appearance of the organs was normal in all groups. Microscopic examination showed that the kidney, liver and intestinal mucosa in animals of all groups was essentially normal. It is interesting to note that the kidney of P.Pe. differs from that of rats and mice by tubules which are considerably longer and which project into the kidney pelvis.

d) Hematology. Hematocrits and blood counts were performed on tail blood. No differences were found in samples obtained from animals which had been slightly anesthetized with ether compared to unanesthized mice nor in samples obtained from the tail vein compared to those obtained by clipping the tail. The results (Table 5) show that P.Pe. fed the seed diet (considered as controls) had a hematocrit of 52% and blood counts of about $11 \times 10^6/mm^3$. P.Ba. had a lower blood count with the same high hematocrit. This may indicate somewhat larger blood cells in this species. These blood values are considerably higher than those found in rats or mice. Comparison of arterial blood with the tail blood and determination of hemoglobin values are now planned. Feeding the semi-purified diets seemed to depress the hemotocrit and increase the blood count. This may, however, be an artifact. We do not trust some of the data obtained in July 1967 because of technical difficulties which we encountered at that time. We nevertheless switched a group of animals from the Gly-7 diet to one which was supplemented with various factors known to be involved in hematopoiesis; iron, vitamin C, vitamin B_{12}, folic acid and methionine (Gly-12). At that time we did not know that this level of methionine had deleterious effects (see below "protein requirements") nor that iron also is not well tolerated. The animals were in bad shape when we measured their hematocrit. We are now testing diets supplemented with vitamin C, vitamin B_{12} and folic acid for any effects on hematological parameters.

e) Adequacy to support growth. The weight gain of groups of laboratory-bred P.Pe. fed different diets was measured: I: mixed seeds; II: semi-purified diet; III: a pelleted mixture of 50% safflower seed and 50% millet (SMD). The starting weight of the animals was between 8-14 g. The exact age at which animals were put on the different diets is not known since we did not wish to jeopardize early growth and survival by weighings. The results are presented in Fig. 1-3. Animals on diets I and II did not grow well until they received supplements of carrots, presumably as a source of moisture. The response of the animals to the semi-purified diet showed considerable individual variations. Of 10 animals, 3 died within 2 weeks, two did so poorly that they were given seeds for 3 weeks after which they did well on the carrot-supplemented diet, 2 did much better after carrot supplementation than before and 3 did fine from the beginning. The effects of carrot supplementation to diet II cannot be assessed from this experiment because it was added after the major growth period of the animals. The final body weight reached by group I and III was greater than that by group II (21.2 g and 18.1 g respectively). Further experiments with larger groups of animals are obviously needed to assess the adequacy of the semi-purified diet for growth.

f) Adequacy of the semi-purified diet for the Kangaroo rat. It seemed worth while to see whether another desert rodent that does not drink water would have a mineral requirement similarly exotic as the pocket mouse. Groups of Kangaroo rats (Dipodomys Mer.) fed either the mixed seed diet (I), the semi-purified diet (II) or SMD (III) are doing well after 5 months. Animals fed the Bell's diet lost weight, although at a slower rate than do P.Pe. Three animals have lost 10g after 4-6 weeks (25% body weight). One died, two are now being supplemented with carrots to see whether this procedure has a similarly salutary effect in this species as it does in the pocket mouse (see above.)

II. Protein requirements:

It seemed interesting to study the response of the mice to different types and levels of proteins. The successful diet described previously contained 22% soy protein. A diet containing 30% soy protein had different effects on P.Pe. than on P.Lo. Five out of a group of 6 P.Pe. did quite well for 10 weeks, although they did not look quite as "healthy" as those on the 22% level. Only 1 out of 4 P.Lo. survived the diet for more than 4 weeks. Attempts to lower the protein level showed that neigher 15% nor 18% was adequate for either species. It therefore appears that for these two species the protein requirement lies in the rather narrow range of 20-25%. This may, however, hold true only for soy protein and only in connection with the other diet ingredients as used by us.

In order to study the reaction of the mice to other proteins commonly used in semi-purified diets we replaced soy protein with casein at a level of 22%. This diet failed after 3-4 weeks. The amino acid composition of casein differs from soy, among others, in a higher level of methionine and a lower level of arginine. Bringing the level of arginine up to that of soy by the addition of 0.7% arginine did not improve the performance of the diet. In order to determine whether the higher level of methionine in casein is the cause of its failure we tried diets containing 22% or 30% soy protein supplemented with 0.5% methionine. Both diets failed although at the lower level of protein 5 out of 7 mice survived about 4 months. This level of methionine appears therefore to have a slow-acting toxic effect. This was also shown when methionine was added to the SMD diet (see above). This diet did not produce any noticable ill effects for about 3 months, but is now failing. We are assuming that the amino acid pattern must be critical for these animals and are now planning additional studies in this area.[1] The casein used in these studies (Nutritional Biochemicals) contains over 2% ash with a considerable amount of phosphorus. It is therefore possible that the failure of casein is due to its mineral content rather than to its unsuitable amino acid composition. Further experiments are obviously needed.

a) Breeding behavior.

1) Manipulation of the environment

We have previously reported that estrus can be induced in P.Pe. during spring and summer months by screen-pairing, e.g. by exposing the females to visual and olfactory contact with a male by means of screen-divided cages (semi-annual progress report, January-June 1967 and manuscript, "Partial contact as a stimulus to laboratory mating in the desert pocket mouse P.Pe.").[2] We have repeated the same type of experiment this fall in order to determine whether the same response can be elicited during the time of year when the mice are normally not reproductively active. Five parous females were tested. The temperature in the laboratory was 65-75° F.

The photoperiod was automatically controlled to correspond to the natural decreasing daylength which was 11.5 to 11 hours during the period of experiment of three weeks. In contrast to our experience last spring, none of the mice showed signs of estrus. We then increased the photoperiod at a rate of about 1 hour/week until it reached 16 hours. Five out of five females came into estrus (Fig. 4) and 3 of them have come into estrus repeatedly. None of a group of 5 parous females which had been exposed to the same schedule of photoperiods but were not screen-paired showed signs of estrus. The cycling females have been mated. It is too early to determine whether they have become pregnant. The results of this experiment indicate that, a) screen-pairing does indeed induce estrus in properly prepared females regardless of natural season, b) preparation includes exposure to a critical photoperiod of approximately 15 hours. This confirms our observations on the incidence of spontaneous estrus in mice exposed to "artificial seasons" in the climatron (semi-annual progress report, January-June 1967).

We are now in the process of investigating in more detail and with a larger group of animals the following questions: What is the critical "long" photoperiod? Is there a critical "short" photoperiod and for how long do mice have to be exposed to it before change to a long photoperiod will produce readiness for estrus? What is the fastest rate of change from a short to a long photoperiod permitting induction of estrus? How long can the mice be kept on the long photoperiod until a short photoperiod has to intervene before resumption of induction of estrus is again possible? Does continued exposure to a male in screen-divided cages eventually lead to refractoriness of the female?

b) Hormone treatments.

1) Females

We have found previously that the gonadotropin Pregnant Mare Serum (PMS) produces in the spring a fertile estrus in P.Pe. A single dose of 6 IU produced estrus in 6 out of 7 females and resulted in 3 litters (semi-annual progres report, January-June 1967). We have repeated this dosage schedule this fall using 10 non-parous females. At the time of treatment 4 mice had been on a constant photoperiod of 14 hours for two months. The other 6 mice had been on a photoperiod which increased about 1 hour/week from 11 hours to 14.5 hours. Two parous mice of the latter group showed a slight external response while none of the former group nor 4 of the latter group reacted at all. These results indicate that 6 IU PMS will produce estrus in P.Pe. only when adequately prepared and that this group was not prepared. In view of our finding that a 15 hour photoperiod may be necessary for preparation when they are to be stimulated by screen-pairing (see a) above), we may have just missed the boat when we used mice during a 14.5 hour photoperiod for the hormone treatment. Further experiments to establish this point will have to wait until financial support can be found.

2) Males

The presence of descendable testes and the length of the penis have been used for selecting males for inducing estrus in females and for breeding. As the day length decreased during this fall the size of the testes decreased in many males to the point where none could be detected by

palpitation. In order to study the effects of Human Chorionic Gonadotropin (HCG) the following 3 groups of males were set up: 5 males exposed to increasing photoperiod (11 hours to 13 hours at 1 hour/week) were injected IP with 5 IU HCG. Five uninjected males exposed to the same increasing photoperiods and 6 males kept at a constant 14 hour day served as controls. All injected males showed a rapid increase in testes size to about half that expected in normal males during spring and summer months. The uninjected mice in increasing photoperiod showed a slow increase in testes to about the same level by the time 15-16 hours light had been reached. The testes of males kept at 14 hours light showed no change during this period. These results indicate that males react positively to stimulation by both HCG and increasing photoperiod.

We have now set up groups of mice in: a) short photoperiod (10 hr.); b) naturally increasing daylength and c) long photoperiod (16 hr.). Each group has been divided into a screen-paired and a singly caged subgroup. Incidence of estrus and cycling will be observed. Results are expected to provide information: 1) whether there is a critical length of photoperiod below which screen-pairing does not induce estrus and how long it is (group a); 2) the length of photoperiod at which estrus occurs without screen-pairing (group b); whether continuous exposure to a long photoperiod and/or a male eventually leads to cessation of cycling.

Footnote[1]: These plans will have to be deferred until a new source of funds can be found.

Footnote[2]: Work in progress.

Summary:

1. The adequacy of a semi-purified diet was tested.

   a) Organ weights, carcass composition, serum electrolytes and organ histology was found to be the same in groups of P.Pe. fed a semi-synthetic diet compared to those fed their usual diet of mixed seeds.

   b) The diet was found adequate to maintain weight and normal behavior in a group of Kangaroo rats, another species of desert rodents that do not require water.

   c) Laboratory-bred P.Pe. required supplementation with moisture containing foods of both the seed and the semi-purified diet for adequate growth.

   d) Study of the hematology of P.Pe. fed the semi-purified diet suggested that this diet may not be fully adequate to maintain normal hematopoiesis.

2. Protein and amino acid requirements of P.Pe. were studied.

   a) A relatively narrow range of 20-25% was found to be required for soy protein in the semi-purified diet.

   b) 22% casein with or without supplementation of arginine did not maintain weight. This failure may be due to the relatively high level of methionine in casein. It was shown that 0.5% methionine added to the soy protein diet or to a pelleted diet composed of safflower meal and millet has a deleterious effect on the mice. The failure of casein may, however, also be due to its high phosphorus and ash content.

3. Breeding behavior was further studied.

   a) "Screen-pairing" of P.Pe. has been shown to induce estrus in properly prepared females regardless of natural season. The preparation included exposure to a critical photoperiod of 14-15 hours.

   b) Treatment of females with PMS did not produce estrus in mice kept at a photoperiod of 14 hours.

   c) Male P.Pe. reacted to either an increasing photoperiod to about 16 hours or to treatment with HCG with increases in the size of testes.

TABLE ONE

Mineral mix, for semi-purified diet for Perognathus

gm per 100 gm mineral mix

	Gly-7[1]	Gly-4[2]
Na-Glycerophosphate	7.5	-
NaCl	-	3.0
KCl	15.0	7.0
K-citrate	-	27.5
Ca-Glycerophosphate	15.0	14.0
Mg-Glycerophosphate	37.0	40.0
$MgSO_4$	7.5	7.5
Fe-citrate	1.0	0.7
Trace minerals as in previous formulas		same
Starch	q.s.	q.s.

[1]Modification as used now.

[2]Original composition.

TABLE 2

Organ weights and carcass composition of Perognathus penicillatus fed different diets[1]

Diet	Body wt. at autopsy	Carcass[2] weight	Moisture[3]	Protein	Fat	Ash	Heart	Kidney	Lung	Liver	Brown Fat
	(g)	(g)	(g/100 g carcass)				(g/100 g body wt.)				
Seeds[4] (I)	21.2±1.1	12.8±0.6	60.7±4.1	19.3±1.3	20.9±4.5	3.3±0.3	0.5±0.04	0.9±0.08	0.6±0.03	3.4±0.4	1.0±0.2
Semi-purified[5] (II)	22.4±1.1	13.4±0.7	58.2±4.3	18.5±1.3	19.9±6.1	2.7±0.2	0.5±0.03	0.9±0.09	0.5±0.06	3.1±0.1	0.7±0.08
Bell's[6] (III)	19.3±0.7	10.9±.4	57.3±2.6	21.2±1.2	16.4±4.0	3.0±0.3	0.5±0.04	1.1±0.02	0.5±0.04	3.3±0.2	0.8±0.2
Same as III plus carrots (IV)	18.3±1.2	9.0±1.3	62.5±1.3	24.8±2.5	10.3±2.7	4.4±0.4	0.4±0.04	1.1±0.04	0.7±0.3	4.0±0.8	0.6±0.09

[1] Four animals (two males, two females) each. Animals in Group I and II had been on diet 2 months; Group III and IV had been on diet 2 weeks. (mean ±SE).

[2] Carcass represents body after removal of head, tail, and all viscera except perirenal fat pads.

[3] Moisture was determined by drying at 110° C to constant weight; fat was determined by Soxhlet extraction of the dried carcass with petroleum ether for 24 hours and weighing of extracted crude fat; proteins and ash were determined on aliquots of the powdered dry, fat-free carcass by the Kjeldahl method of nitrogen determination and ashing at 800° C respectively.

[4] Equal parts of sunflower seeds, millet, canary seeds and oats.

[5] Composition of diet has been given in semi-annual report, January-June 1967.

[6] Composition see Semi-Annual Report, January-June 1966.

TABLE 3

Carcass composition of male and female P.Pe. fed different diets[1]

(g/100g Carcass)

	Moisture		Fat		Protein	
	Male	Female	Male	Female	Male	Female
Seeds (I)[2]	65.7	53.3	9.7	24.3	20.7	17.9
Semi-purified (II)	63.7	50.6	10.4	29.3	20.5	16.4
Bell's (III)	54.9	52.1	12.4	24.3	22.3	18.6
Bell's + Carrot (IV)	63.8	61.1	6.2	14.4	23.0	21.5

[1] Two animals of each sex on each diet. Group I and II had been on diet. 2 months, Groups III and IV had been on diet 2 weeks.

[2] See footnotes to Table 2 for diet compositions.

TABLE 4

Concentration of major electrolytes in blood of P.Pe. fed different diets[1]

	Na^2	K^2	Mg^3	Ca^3
	mequ/100 ml serum			
Seeds (I)[4]	15	0.44	0.22	0.49
Semi-purified (II)	16	0.44	0.24	0.53
Bell's (III)	15	0.43	-	-
Bell's + Carrots (IV)	15	0.50	-	-

[1] Groups I and II had been on diet 2 months; Group III and IV 2 weeks.
[2] Na, K were determined within a flame photometer.
[3] Mg, Ca were determined by a micromethod utilizing atomic absorbtion.
[4] See footnotes to Table 2 for diet composition.

TABLE 5

Hematology of P.Pe. and P.Ba. fed different diets

Diet	Number of animals	Time on diet	Method[1] of bleeding	Species[2]	Packed cell volume %	RBC x10^6/mm^3
Seeds	5[3]	8 W	C	P.Pe.	52	-
	4[4]	20 W	V	same	52	11.5
	5[5]	32 W	V	same	53	11.0
Gly-7[7]	5[3]	10-15 W	C	same	44	-
	2[4]	23 W	V	same	48	13.2
	7[6]	14 W	V	same	52	12.5
Gly-12[8]	7[4]	6-8 W	C	same	50	-
Gly-7 + carrot	2[3]	6 W	C	same	47	-
Gly-7	4[6]	31 W	V	P.Ba.	52	10.1

[1] Blood was obtained from the tail vein (V) or by clipping tail (C).
[2] P.Pe. = P. Penicillatus; P.Ba. = P. Bailey.
[3] Experiment done July 1967.
[4] " " October 1967
[5] " " January 1968
[6] " " November 1967
[7] Semi-purified diet. Composition see previous Semi-Annual Report.
[8] " " " Composition the same as Gly-7 with the addition of 0.5% methionine and supplemented with iron, vitamin C, vitamin B_{12} and folic acid.

FIGURE 1 : WEIGHT GAIN OF P. PE. ON SEED DIETS

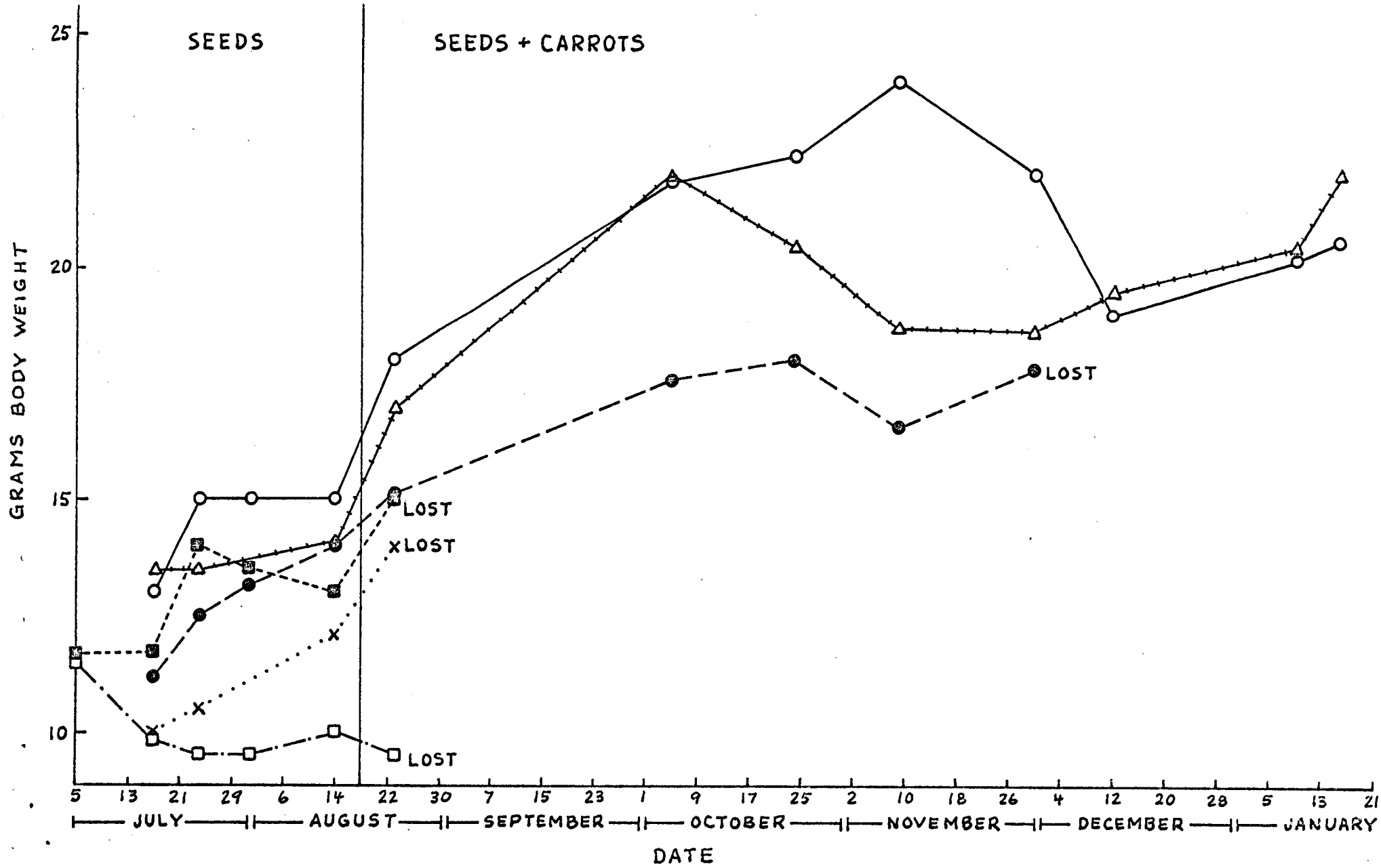


FIGURE 2: WEIGHT GAIN OF P. PE. ON SMD DIETS

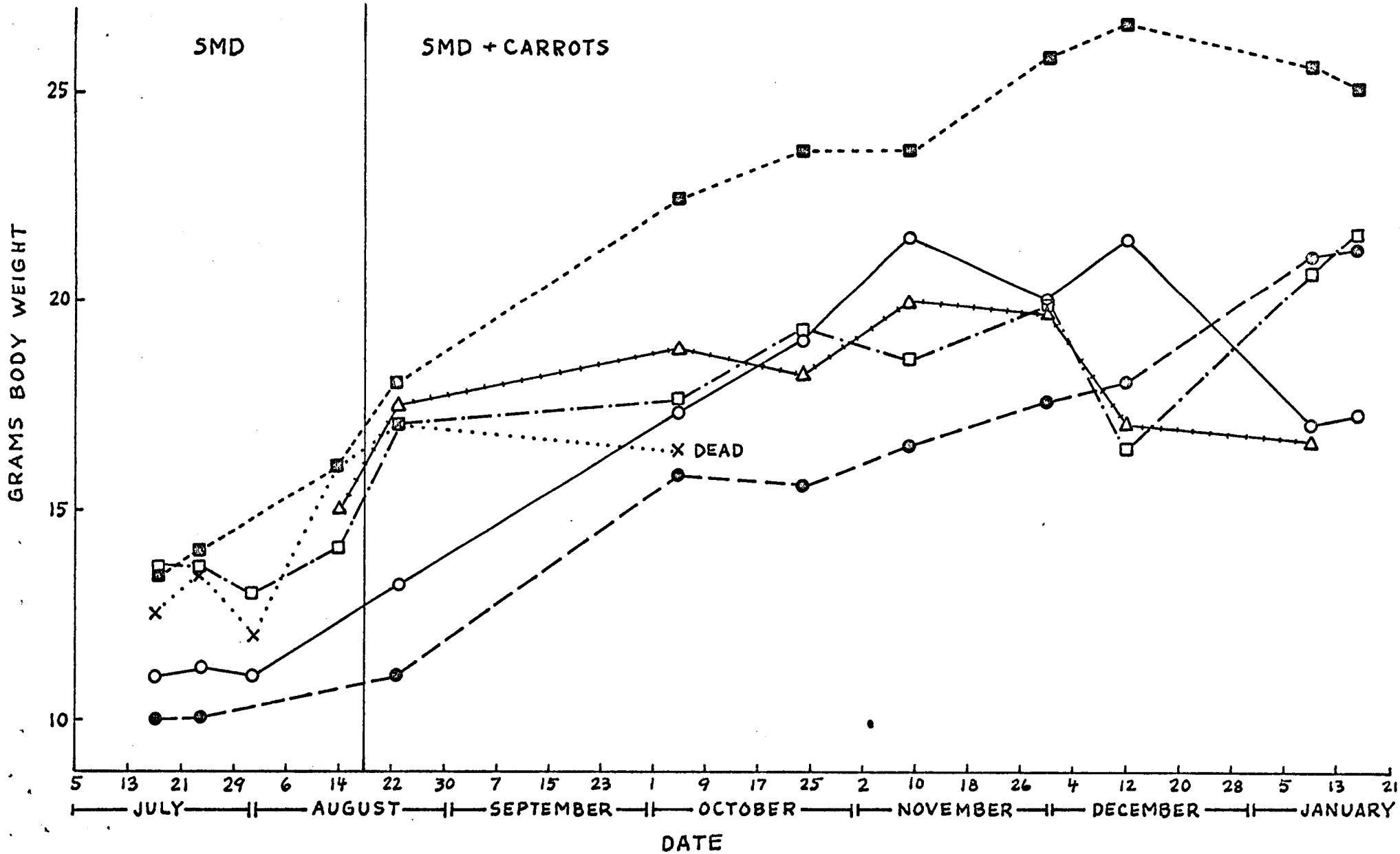


FIGURE 3: WEIGHT GAIN OF P. PE. ON SEMIPURIFIED DIETS

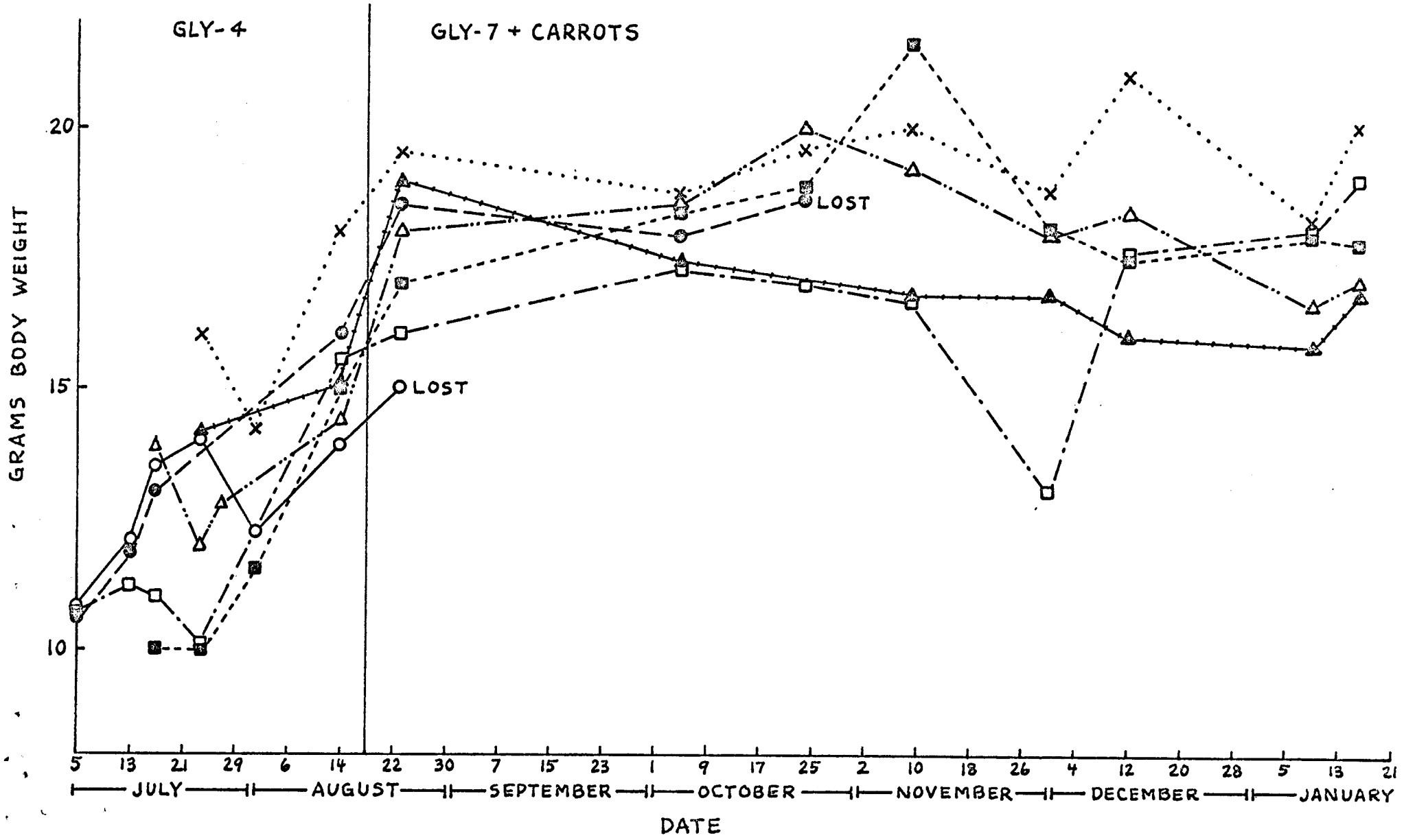
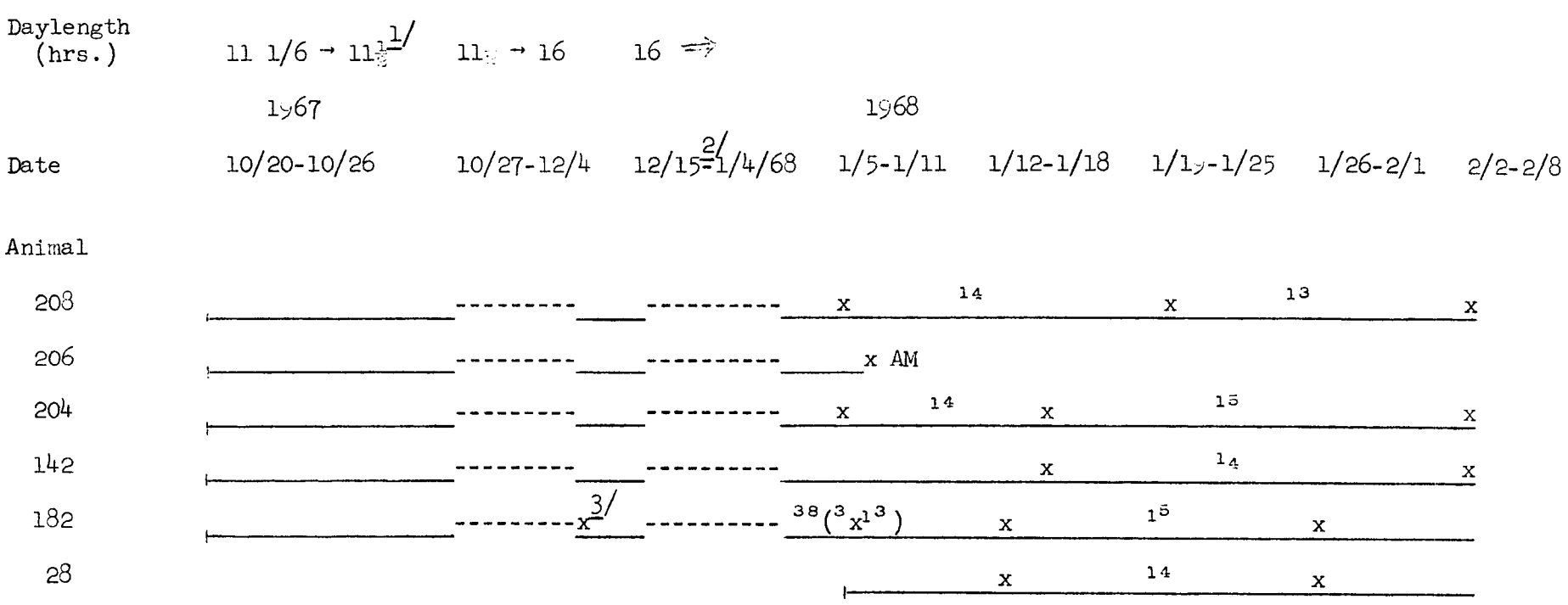


FIGURE 4

Incidence of estrus in screen-paired P.Pe. in response to photoperiod



Legend:

_____	Female screen-paired.
x	Estrus observed.
x y x	Days between estrus.
AM	Screen removed, attempted mating.
1/	Photoperiod increased approximately 0.5 hours/5 days.
2/	Animals were not checked for estrus 12/15-1/3.
3/	Estrus observed 12/6 at photoperiod of 15½ hours.